Genet. Res., Camb. (1918), **38**, *pp.* 47–55
With 1 *plate and* 1 *text-figure*
Printed in Great Britain

A gene (*Neu–1*) on chromosome 17 of the mouse affects acid α-glucosidase and codes for neuraminidase

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(*Received* 13 *November* 1980 *and in revised form* 19 *December* 1980)

SUMMARY

An electrophoretically detectable variant of acid α-glucosidase has been found in SM/J mice. This variant is attributable to excess sialylation of the enzyme and is determined by a gene, alpha-glucosidase processing, *Aglp*, on chromosome 17. In addition, as also reported by Potier, Lu Shun Yan & Womack (1979), SM/J mice are relatively deficient in neuraminidase and it appears that the low level of this enzyme in SM/J is determined by an autosomal codominant gene, neuraminidase-1, *Neu-1*. Preliminary data indicate that *Neu-1* is also on chromosome 17. It seems probable that the several processing genes *Apl*, *Aglp* and *Map-2* which are all closely linked on chromosome 17 are one and the same, a gene *Neu-1* coding for neuraminidase.

1. INTRODUCTION

A number of genes have been identified in the mouse, *Mus musculus*, which determine differences in the degree of sialylation of enzymes which are glycoproteins. These include alpha-mannosidase processing-1 (*Map-1*) and alpha-mannosidase processing-2 (*Map-2*) which influence the sialylation of α-mannosidase (EC 3.2.1.24) (Dizik & Elliott, 1977, 1978) and acid phosphatase-liver (*Apl*) which affects acid phosphatase (EC 3.1.3.2) (Lalley & Shows, 1977). *Map-1* is on chromosome 5 and *Map-2* and *Apl* have been assigned to the same region of chromosome 17 (Dizik & Elliott, 1977, 1978; Womack & Eicher, 1977). The inbred strain SM/J is exceptional for it carries different alleles at *Map-2* and *Apl* from those found in other inbred strains tested.

We have observed a variant electrophoretic phenotype of another enzyme which is a glycoprotein, acid α-glucosidase (EC 3.2.1.20), in SM/J mice and again the variation is attributable to excess sialylation. Breeding studies have shown that a gene on chromosome 17 closely linked to *Apl* determines this phenotype. This gene is designated alpha-glucosidase processing (*Aglp*).

In man, individuals deficient in neuraminidase (*N*-acetyl neuraminic acid hydrolase, EC 3.2.1.18), who have the lysosomal storage diseases classified as the sialidoses and mucolipidoses show abnormal sialylation of a number of glycoprotein enzymes (Champion & Shows, 1977; Swallow *et al.* 1979; Thomas *et al.* 1979). We show, as Potier *et al.* (1979) also find, that SM/J mice are relatively deficient in neuraminidase, and furthermore we propose that a single gene on chromosome 17 of the mouse determining neuraminidase can affect acid α-glucosidase, acid phosphatase and α-mannosidase.

2. MATERIALS AND METHODS

(i) *Mice*

Mice of strains AKR, BALB/c/By, BALB/c/Gr, C3H/HeH, C3H/He/Lac, C57BL/Gr, DBA/1, F/St, IS/Cam, NMRI/Lac, NZW, SM/J, SWR/J were tested as well as 15 mice of the Peru-Coppock stock, which has been recently derived from the wild (Wallace, 1976) and 20 wild caught animals from Skokholm island.

(ii) *Sample preparation*

Mice were killed by cervical dislocation. For electrophoretic studies of acid phosphatase and acid α-glucosidase tissue extracts were made using the proportion 1 gram of fresh or frozen tissue to 2 grams of distilled water followed by homogenisation in a Silverson homogeniser. Homogenates were centrifuged at 2000 rev/min (MSE Mistral 6L centrifuge) for 15 min. Supernatants were stored at −20 °C for up to two months. For quantitative studies of neuraminidase activity tissue extracts were prepared in the same way except that fresh tissues and fresh supernatants were always used.

For electrophoretic studies of phosphoglycerate kinase testis extracts were made using the proportion 1 g of fresh or frozen testis to 5 g of Tris/EDTA/citric acid buffer pH 7·5 of Ratazzi *et al.* (1967). The homogenates were diluted one part to six parts buffer, centrifuged at 3000 rev/min (MSE Super Minor) for 5 min and the supernatants were used immediately for electrophoresis.

Fibroblasts were cultured from small pieces of skin and lung of C3H/HeH and SM/J mice as described by Swallow *et al.* (1975). After 4–6 passages the cells transformed spontaneously.

Treatment with bacterial sialidase was carried out as described previously (Swallow *et al.* 1977).

(iii) *Electrophoresis*

Electrophoresis of acid α-glucosidase and acid phosphatase was done using starch gels with incorporated maltose as described by Swallow *et al.* (1975). After electrophoresis acid α-glucosidase was detected as described in that paper and acid phosphatase as described by Swallow & Harris (1972).

Electrophoretic analysis of phosphoglycerate kinase was carried out as described by Johnston & Cattanach (1981).

(iv) *Assay of Neuraminidase*

Neuraminidase assays were carried out using 4-methylumbelliferyl-α-D-*N*-acetylneuraminic acid (Koch-Light Laboratories Ltd) as described previously (Winter *et al.*, 1980).

3. RESULTS

(i) *Electrophoretic variation of acid α-glucosidase*

When acid α-glucosidase was examined after electrophoresis of liver extracts the enzyme from SM/J mice had greater anodal electrophoretic mobility (AGLP-A) than that found in all other mice tested, which were of phenotype AGLP-B (Plate 1*a*). When liver extracts were treated with neuraminidase prior to electrophoresis the mobility of the enzyme from SM/J was retarded so that this strain now appeared to have the AGLP-B phenotype. Treatment with neuraminidase did not affect the mobility of acid α-glucosidase from any other mice tested (Plate 1*a*).

Although enzyme activity was also detectable in spleen, cultured fibroblasts, brain, heart and kidney only the spleen of SM/J mice showed the variant phenotype characteristic of liver. In the other tissues the mobility of the enzyme from SM/J was less anodal than in liver, and had similar mobility to that found in livers from other mice. No enzyme activity was observable in lymphocytes.

(ii) *Developmental studies*

Electrophoresis of acid phosphatase and acid α-glucosidase in liver extracts from mice ranging in age from 11 days' gestation to adulthood was carried out. Even as early as fourteen days gestational age activity of both enzymes was detected (Plate 1*b*).

The electrophoretic pattern of both enzymes in an 11 day C3H/HeH foetus resembled that seen in the adults, but developmental changes in electrophoretic mobility were found in SM/J mice. Thus in a 14 day SM/J foetus (the earliest stage tested) the acid α-glucosidase phenotype was similar to that found in C3H/HeH mice, so that the SM/J mouse at this stage had the AGLP-B phenotype. As development proceeded a gradual shift in electrophoretic mobility of acid α-glucosidase was seen but the AGLP-A pattern found in the adult was not present until 8 weeks after birth. Parallel changes were also noticed for acid phosphatase. At the earliest stages tested differences in electrophoretic mobility between liver acid phosphatase in C3H/HeH and SM/J were not observed, and the acid phosphatase pattern seen in adult SM/J was not present till 8 weeks after birth.

(iii) *Genetics*

Liver extracts of (C3H/HeH × SM/J)F_1 mice all showed the AGLP-B phenotype found in the C3H/HeH parent (Plate 1*a*). When mice resulting from the backcross of the F_1 to the SM/J parent were tested two phenotypic classes were

https://doi.org/10.1017/S0016672300020395 Published online by Cambridge University Press

found, one having the AGLP-A phenotype like SM/J and the other the AGLP-B phenotype like the F_1 parent. 86 animals had the AGLP-A phenotype and 102 the AGLP-B phenotype. Nineteen animals resulting from the backcross of the F_1 to the C3H/HeH parent were also tested and all had the AGLP-B phenotype. When F_2 animals were tested 3 showed the AGLP-A phenotype, and 15 showed the AGLP-B phenotype. These data provide evidence that the difference in electrophoretic

Table 1. *Linkage of Aglp and Apl*

Cross: $\frac{Aglp^a\ Apl^a}{Aglp^b\ Apl^b} \times \frac{Aglp^a\ Apl^a}{Aglp^a\ Apl^a}$

Gamete	Number	Crossover
$Aglp^a\ Apl^a$	86	None
$Aglp^b\ Apl^b$	102	None
	188	

Table 2. *Linkage of Aglp, Apl and Pgk-2*

Cross: $\frac{Aglp^a\ Apl^a\ Pgk\text{-}2^a}{Aglp^b\ Apl^b\ Pgk\text{-}2^b} \times \frac{Aglp^a\ Apl^a\ Pgk\text{-}2^a}{Aglp^a\ Apl^a\ Pgk\text{-}2^a}$

Gamete	Number	Crossover
$Aglp^a\ Apl^a\ Pgk\text{-}2^a$	54	None
$Aglp^b\ Apl^b\ Pgk\text{-}2^b$	73	None
$Aglp^a\ Apl^a\ Pgk\text{-}2^b$	0	None
$Aglp^b\ Apl^b\ Pgk\text{-}2^a$	1	*Aglp Apl, Pgk-2*
	128	

mobility of liver acid α-glucosidase found between SM/J and other inbred strains is determined by a single gene. We therefore suggest the gene name α-glucosidase processing (*Aglp*) and that the allele carried by SM/J be designated the $Aglp^a$ allele and that present in all other inbred strains so far tested the $Aglp^b$ allele.

(iv) *Linkage*

In the cross (C3H/HeH × SM/J)F_1 × SM/J *Aglp*, *Apl*, *Map-2* and *Pgk-2* were segregating. *Map-2* was discarded as a marker because new phenotypes which were not seen in the parental mice were found in some of the offspring, possibly because more than one gene was segregating, presumably *Map-1* as well as *Map-2*, but we have not tested this. *Pgk-2* determines the testis form of phosphoglycerate kinase and so was scored only in mature males. No recombinants were found between *Aglp* and *Apl* in 188 offspring tested and only one offspring was a recombinant for *Aglp* and *Pgk-2* (Tables 1 and 2).

(v) *Quantitative studies*

Neuraminidase levels were measured in livers from C3H/HeH, SM/J (C3H/HeH × SM/J)F_1, and the backcross (C3H/HeH × SM/J)F_1 × SM/J. The results are shown in Table 3 and Fig. 1. Livers from SM/J are relatively deficient in

Table 3. *Neuraminidase activity in mouse liver*

C3H/HeH	(C3H/HeH × SM/J)F_1	SM/J
4·27 ± 0·55 (3)	2·36 ± 0·26 (4)	0·86 ± 0·25 (5)

Neuraminidase activity expressed as *n* moles 4-methylumbelliferone produced per hour per milligram protein at 37 °C. ± S.D. Figures in brackets refer to the number of mice used.

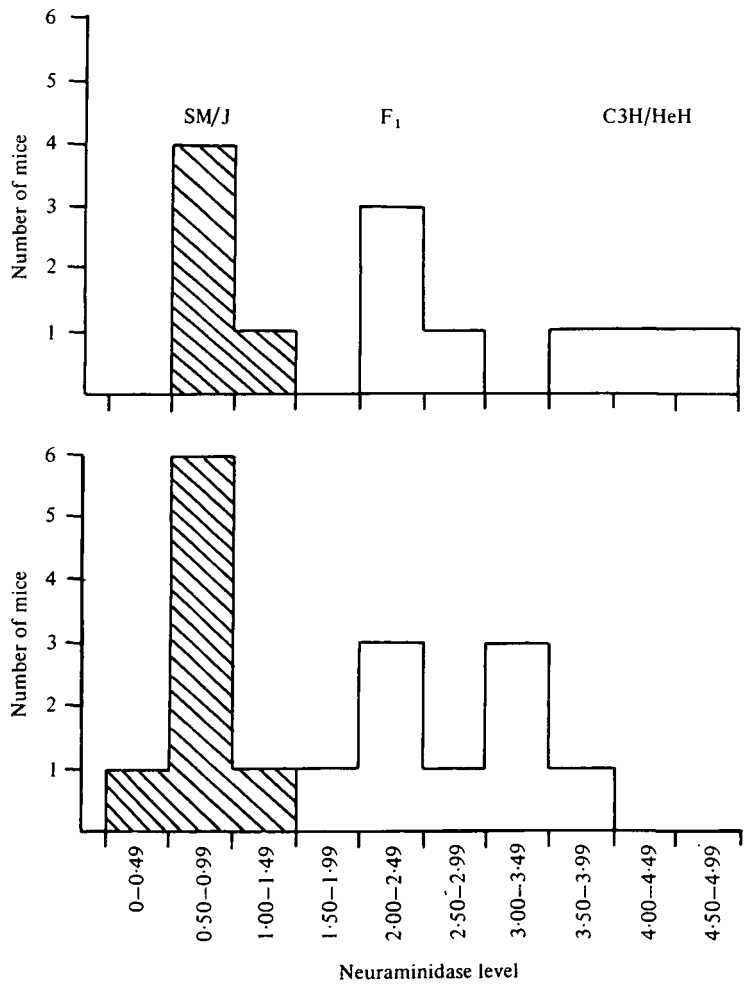

Fig. 1. Distribution of neuraminidase activities and AGLP phenotype in C3H/HeH, SM/J, (C3H/HeH × SM/J)F_1, and in mice resulting from the backcross (C3H/HeH × SM/J)F_1 × SM/J. Enzyme levels measured as n moles 4-methylumbelliferone produced/h/mg. protein at 37 °C. The shaded portion represents mice with the AGLP-A phenotype and the unshaded part those with the AGLP-B phenotype.

https://doi.org/10.1017/S0016672300020395 Published online by Cambridge University Press

neuraminidase, containing approximately 20 % of the activity found in C3H/HeH. Intermediate levels are found in F_1 offspring and a wide range of activity levels was found in the mice resulting from the backcross. When the backcross mice were classified for AGLP it was clear that the neuraminidase levels fell into two classes according to the AGLP phenotype. One class with the AGLP-A phenotype had low activity of neuraminidase, ($\bar{x} = 0{\cdot}84 \pm 0{\cdot}23$) comparable with that found in SM/J, and the other of phenotype AGLP-B had neuraminidase levels comparable with those found in F_1 individuals ($\bar{x} = 2{\cdot}70 \pm 0{\cdot}66$).

(vi) *Pathology of SM/J mice*

SM/J mice were examined for clinical features similar to those characteristic of human neuraminidase deficiency. The features in man include progressive loss of visual acuity due to perinuclear cataracts and cherry red spots in the macula, bony abnormalities, mental deterioration and myoclonus (Lowden & O'Brien, 1979). SM/J mice ranging in age from two months to fifteen months were examined and no abnormalities were found, except that in three animals aged between 12 and fifteen months examination of the eye with a slit lamp and an opthalmoscope revealed the presence of age-associated lens opacities. Preliminary histological examination of various tissues from these mice showed no significant difference from normal.

4. DISCUSSION

The difference in electrophoretic mobility of liver acid α-glucosidase in SM/J compared to other inbred strains can be accounted for by an increase in the degree of sialylation of the enzyme. This was shown by the effect of treatment of the samples with bacterial neuraminidase prior to electrophoresis. This is not peculiar to acid α-glucosidase since genetically determined differences in the degree of sialylation of α-mannosidase and acid phosphatase have also been found (Dizik & Elliott, 1977, 1978; Lalley & Shows, 1977). A preliminary report has also appeared of a similar variant phenotype of aryl sulphatase in SM/J mice (Henthorn & Daniel, 1978).

The results of the genetic tests indicate that the difference in electrophoretic mobility is controlled by a single gene. The dominance of the *Aglp*^{b} allele (found in C3H/HeH) shows that this variation is not caused by a mutation in the structural gene for α-glucosidase. Further support for this view comes from studies of tissue distribution and development; for although the enzyme can be detected in foetal liver, the variation is not clearly apparent until mice are 8 weeks old, and although the enzyme is present in many tissues the variant phenotype can be seen only in adult liver and spleen. The most likely interpretation of these findings is that the product of the gene *Aglp* modifies the α-glucosidase protein after it has been synthesized and does this by controlling the extent of sialylation of the enzyme.

The recombination frequency between *Aglp* and *Pgk-2* is $0{\cdot}8\,\% \pm 0{\cdot}8$ (at the 95 % confidence level the genes are 0·02 to 4·3 cM apart) indicating that *Aglp* is on chromosome 17. The genetic studies also show that the *Aglp* gene is closely

linked to *Apl*. Thus at the 95% confidence level *Apl* and *Aglp* are 0 to 1·57 cM apart. Dizik & Elliott (1978) found no recombinants between *Apl* and *Map-2* in 88 backcross offspring and Womack, Lu Shun Yan & Potier (1981) found none in a further 51 backcross and 52 F_2 offspring. Since these three genes exert similar effects, it seems probable that they are one and the same gene, controlling the degree of sialylation of some glycoprotein enzymes.

As neuraminidase deficiency in man had been shown to cause similar phenotypic changes to a range of glycoprotein enzymes (Champion & Shows, 1977; Swallow *et al.* 1979; Swallow *et al.* 1981), neuraminidase levels were measured in SM/J and C3H/HeH. The SM/J mouse liver was shown to be relatively deficient in neuraminidase activity, having 20% of the activity found in C3H/HeH mice. While this work was in progress similar findings were made by Potier *et al.* (1979) who found that SM/J mice had 15% of normal activity. Potier *et al.* (1979) also showed that other tissues such as kidney were not deficient.

We have shown that heterozygotes have intermediate activity indicating that neuraminidase deficiency is a primary effect. Thus our interpretation is that a gene neuraminidase-1, *Neu-1*, on chromosome 17 linked to *Aglp* and *Apl* determines neuraminidase activity. This agrees with the finding of Womack & Potier (1979); Womack *et al.* (1981) who found that the congenic strain B.10.SM(70NS)/Sn, in which a region of SM/J chromosome 17 including the H-2 complex has been backcrossed on to C57BL/6J, was also deficient in neuraminidase activity, imputing responsibility to chromosome 17. The most probable explanation of all these findings is that the gene determining high and low levels of activity of neuraminidase on chromosome 17 of the mouse is the same gene as that affecting the level of sialylation of α-glucosidase, acid phosphatase and α-mannosidase. Very recent work of Womack *et al.* (1981) confirms this suggestion. This would imply that neuraminidase plays a role in the processing of these enzymes.

It is of some interest that *Apl* appears to show additive expression of alleles (Lalley & Shows, 1977) whereas *Map-2* and *Aglp* show dominance and in each case the dominant allele is the one that leads to the less sialylated form of the enzyme. A second gene *Map-1* on chromosome 5 affects the sialylation of α-mannosidase and possibly also acid phosphatase (Dizik & Elliott, 1977; R. W. Elliott cited by Paigen, 1979). *Map-1* also shows dominance but in this case the excessively sialylated form is dominant. After carrying out the experiment which demonstrated linkage of *Apl*, *Aglp* and *Pgk-2* we carried out a second linkage test to try to establish the gene order on chromosome 17 between *Apl* and *Pgk-2* and kidney catalase, *Ce-2*. In this experiment (C3H/HeH × SM/J)F_1 females were crossed to C3H/HeH males instead of SM/J males which were used in the first test. Interestingly no evidence of linkage between *Apl* and the other two genes was found and close examination of the *Apl* phenotypes revealed the existence of new phenotypes in this particular backcross but these could not be typed with confidence (Peters & Swallow unpublished). One explanation of these results is that we were observing the segregation of a second gene affecting the sialylation of acid phosphatase, possibly *Map-1*.

Low levels of neuraminidase do not appear to be associated with any clinical

features in the SM/J mouse. However the levels of residual activity are substantially higher than found in humans with neuraminidase deficiency, and whereas in the mouse the deficiency is restricted to liver, in man it is widespread. Potier *et al.* (1979) have identified two types of neuraminidase in the mouse which hydrolyse 4-methylumbelliferyl *N*-acetylneuraminic acid, a stable and a labile form and it appears that the SM/J liver is deficient in the labile component. Although it seems that the deficiency of neuraminidase in SM/J mice is a primary effect, the exact nature of the defect is unclear.

We thank Paul Walding for his help in studying the pathology of SM/J mice, Lynne West for help with the neuraminidase assays and Mr E. Vickers of Koch-Light Laboratories Ltd. for providing the substrate.

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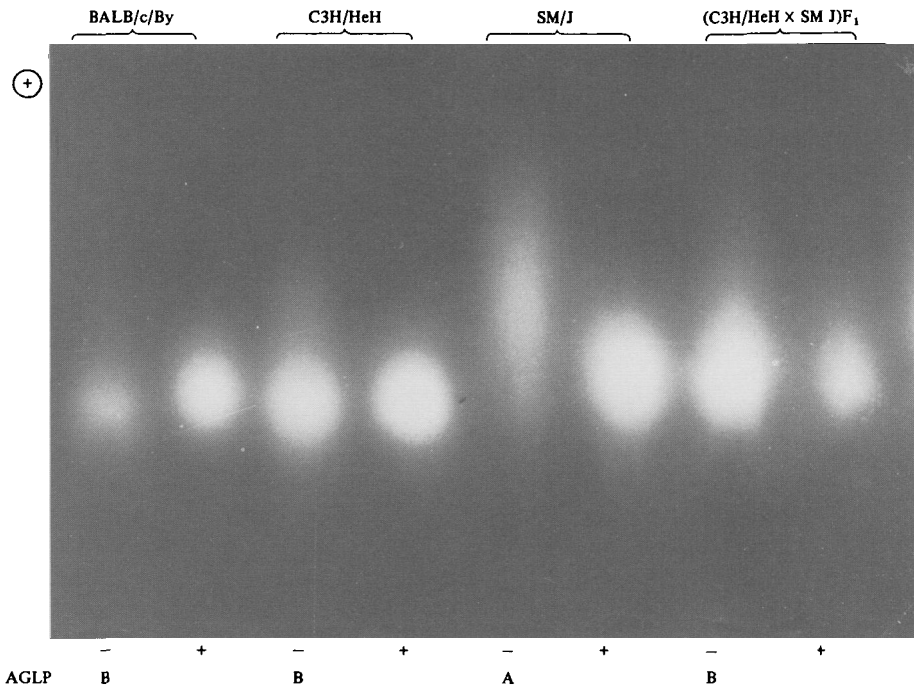

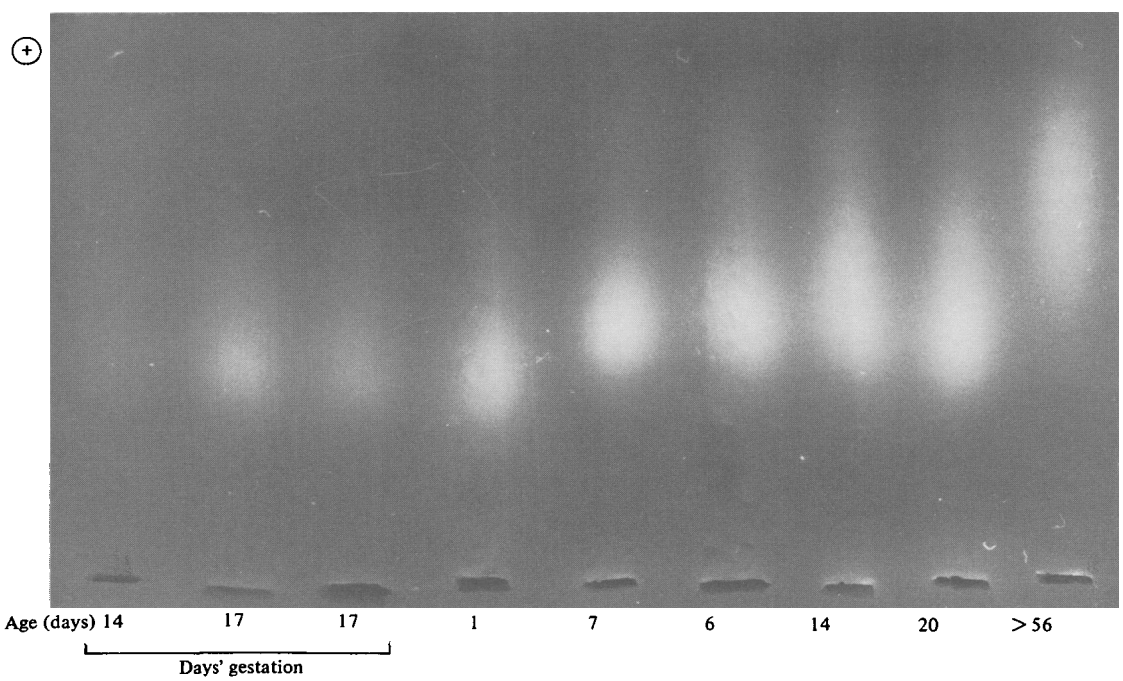

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PLATE 1

(*a*) Photograph of starch gel showing acid α-glucosidase from mouse liver extracts with (+) and without (−) treatment with bacterial neuraminidase.
(*b*) Photograph of starch gel showing SM/J liver acid α-glucosidase during development. The ages of the mice ranged from 14 days' gestation to adult.